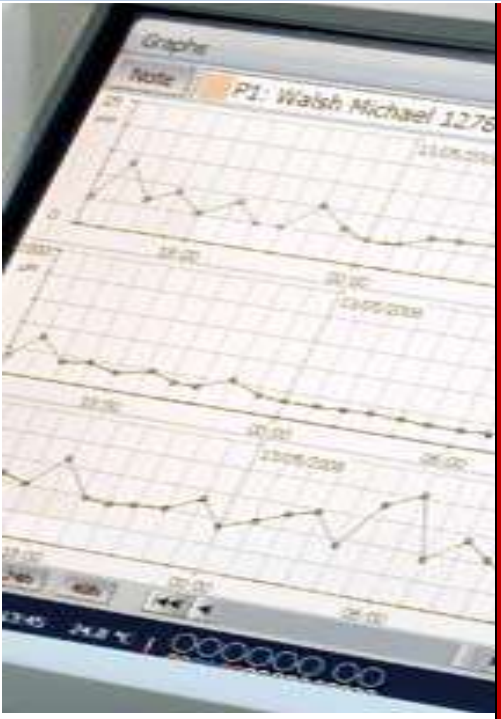
Neurochemical
Monitoring
in the
Injured Brain

MICRODIALYSIS in Neurointensive Care

Urban Ungerstedt



Microdialysis in Neurointensive Care

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SUMMARY

Microdialysis is a technique for continuous sampling of the interstitial fluid chemistry of tissues and organs. It is minimally invasive and simple to perform in a clinical setting. The microdialysis catheter samples all small molecular substances present in the interstitial fluid, however, the use of microdialysis in neurointensive care has focused on markers of ischemia and cell damage.

Lactate/Pyruvate ratio (LPR) is a marker of changes in the redox state of cells and a ratio >25 is an early warning of ischemia and mitochondrial dysfunction.

Glycerol is a marker of cell membrane decomposition. Loss of energy due to ischemia and/or mitochondrial dysfunction eventually leads to an influx of calcium and a decomposition of cell membranes, which liberates glycerol into the interstitial fluid.

Brain glucose is an important marker due to the increasing interest in controlling blood glucose within defined limits. Low systemic glucose may cause brain hypoglycemia during neurointensive care leading to secondary brain damage.

Glutamate may open neuronal calcium channels initiating a pathological influx of calcium thus provoking cell damage. However, most likely an increase in extracellular glutamate signals an energy deficiency causing a decreased uptake of glutamate into astrocytes.

The Penumbra, i.e. tissue adjacent to a focal lesion, is considerably more vulnerable than normal brain tissue. A microdialysis catheter positioned in the penumbra detects early signs of ischemia and mitochondrial damage that may lead to cell damage. After SAH a microdialysis catheter in the tissue at risk will detects vasospasm hours before clinical signs are evident.

Microdialysis predicts outcome in SAH, TBI and MCA patients and is used increasingly as a tool to individualize patient treatment in routine neurointensive care.

INTRODUCTION

This text focuses on microdialysis during neurointensive care. The emphasis is on the use and interpretation of bedside microdialysis in clinical practice. I have highlighted those facts where there is unanimous agreement on findings as well as interpretations. The intention is to present knowledge that is useful for preventing and relieving secondary insults, predicting outcome and guiding therapy during neurointensive care.

THE MICRODIALYSIS TECHNIQUE

Microdialysis is a technique for sampling the chemistry of the interstitial fluid of tissues and organs. It started as an animal research technique (Ungerstedt and Pycock 1974) but has gradually been applied to man as well as animal (Ungerstedt 1991). It is minimally invasive and simple to perform in a clinical setting. It has become a standard technique in physiological and pharmacological investigations on animals with about 15.000 published papers. During the last 10 years it has developed into a clinically useful technique with close to 3000 papers published on the use in brain and peripheral tissues.

In its simplicity a microdialysis catheter forms a “biosensor” where samples of the tissue chemistry are transported *out of the body* for analysis in contrast to the traditional biosensor where the analysis takes place *inside the body*. The availability of modern analytical techniques has made microdialysis a “universal” biosensor capable of monitoring essentially every small and medium sized molecular compound in the interstitial fluid of endogenous as well as exogenous origin. Today, there are CE labeled and FDA cleared microdialysis catheters (Fig 1) and bedside chemical analyzers (Fig. 11) available for human use on the world market (M Dialysis AB, Solna, Sweden).

The dialysis membrane at the distal end of a microdialysis catheter functions like a blood capillary. Chemical substances from the interstitial fluid diffuse across the membrane into the perfusion fluid inside the catheter. The *recovery* of a particular substance is defined as the concentration in the dialysate expressed as a percent of the concentration in the interstitial fluid.

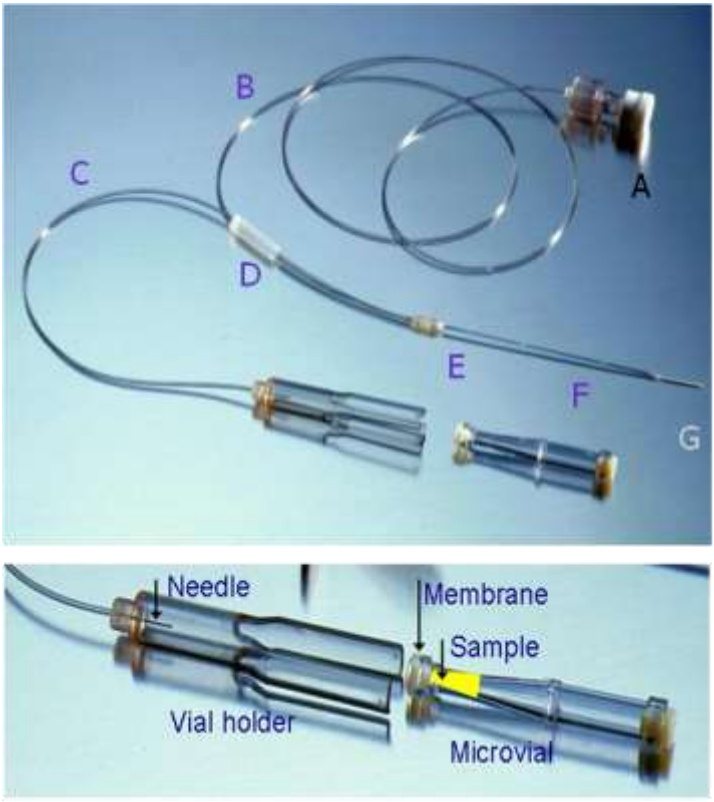

Fig 1. The 70 Brain MD catheter. The luer (A) connects to the106 Pump Syringe. The inflow (B) and outflow (C) tubes are surrounded by the sliding cuff (D) which is used to suture the catheter to the skin of the scalp. The two tubes join in the cylindrical “liquid cross” (E), which connects to the shaft (F) and the dialysis membrane (G) with its gold tip. The protective tube is not shown. The Vial holder and the Microvial are shown in the lower panel. The needle of the holder penetrates the membrane of the Microvial when the vial is pushed into the holder. The sample is collected in the neck of the vial just under the membrane.

A low perfusion flow and a long dialysis membrane give a high recovery. If the membrane is long enough and the flow slow enough, the concentration in the dialysate will approach the concentration in the interstitial fluid, i.e. recovery will be close to 100%. In the human brain the common perfusion flow is 0.3 µl/min and the length of the membrane

usually 10 mm (allowing exact positioning in relation to e.g. a lesion). Under these conditions the recovery has been estimated to be approximately 70% (Hutchinson *et al.,* 2000). By using a longer membrane, e.g. 30 mm, and the same perfusion flow, it is possible to reach 100% recovery in the human brain.

It is important to realize that the concentration in the dialysate not only depends upon the flow and the length of the membrane, but also upon the supply of substances from blood capillaries as well as uptake and release from cells. For example, the supply of glucose to the microdialysis catheter may decrease due to a decrease in the capillary blood flow or due to an increase in the cellular uptake of glucose (Fig 2).

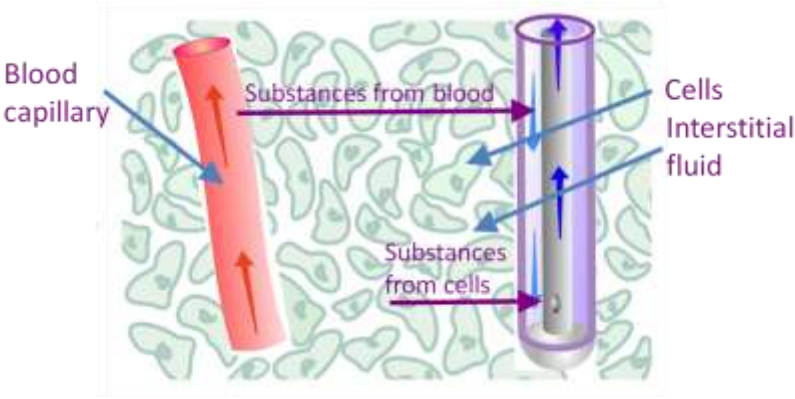

Fig 2. Principles of microdialysis. The microdialysis catheter takes up substances delivered by the blood e.g. glucose and drugs, but also substances released from the cells e.g. markers of cellular metabolism. Substances may also be introduced into the tissue by including them in the perfusate, e.g. precursors of enzymatic processes, and the products of the process may then be recovered by the microdialysis catheter. Another unique possibility is to deliver drugs to the tissue for studies of pharmacodynamic effects or for obtaining local therapeutic effect. Treatment of brain tumors have been attempted using this approach.

The high recovery of substances that can be achieved in the human brain makes it possible to analyze most neurotransmitters and energy metabolites but also cytokines (Hillman *et al.,* 2005) and small proteins using catheters with a cut off of 100 kDa as compared to the conventional catheter with 20 kDa cut off. The two catheters give

equivalent results for small molecular substances such as glucose, lactate, pyruvate, glutamate and lactate/pyruvate ratio. The high cut off microdialysis catheters can, therefore, be used for routine clinical monitoring of extracellular substances (Hutchinson *et al.,* 2005), as well as for research on e.g. larger molecular weight peptides.

BIOCHEMICAL MARKERS OF ISCHEMIA AND CELL DAMAGE

The interstitial fluid is the "cross road" of all substances passing between cells and blood capillaries. By monitoring this compartment in the brain it is possible to get crucial information about the biochemistry of neurons and glia and how seriously brain cells are affected by for example hypoxia, ischemia, mitochondrial dysfunction, hyperemia, trauma, hemorrhage, vasospasm as well as various physiological, pharmacological and surgical interventions during intensive care.

Although microdialysis recovers essentially all small molecular substances present in the interstitial fluid the use of microdialysis in neurointensive care has focused on markers of ischemia, mitochondrial dysfunction and cell damage. The reason is that they are of obvious importance for the survival of the tissue, well understood from a biochemical point of view and easy to interpret in the clinical setting of intensive care.

Microdialysis tells us how cells react to an increase or decrease in the supply of oxygen and glucose. However, while normal brain tissue may not suffer when exposed to a moderate decrease in oxygen and glucose, vulnerable cells in the peri-contusional penumbra may simply not survive. *In this way severe secondary damage to brain tissue may pass unnoticed if microdialysis is not performed in the most vulnerable tissue of the brain* (see below).

LACTATE/PYRUVATE RATIO

The lactate/pyruvate ratio is a well-known marker of changes in the redox state of cells caused by e.g. ischemia and mitochondrial dysfunction. Pyruvate is formed from glucose in the anaerobic part of the glycolysis generating 2 molecules of ATP. It enters the citric acid cycle provided that oxygen is available. The citric acid cycle is the dominant producer of energy yielding 32 molecules of ATP. If the tissue

is exposed to ischemia, i.e. a decrease in blood flow causing an inadequate supply of oxygen and glucose, the production of ATP from the citric acid cycle decreases.

The cells attempt to compensate for the decrease in ATP production by increasing the turnover of glucose in the anaerobic part of the glycolysis. During this process it is necessary to regenerate NAD^+ from NADH by converting pyruvate to lactate, which causes an increase in lactate and the lactate/pyruvate ratio (Fig 3).

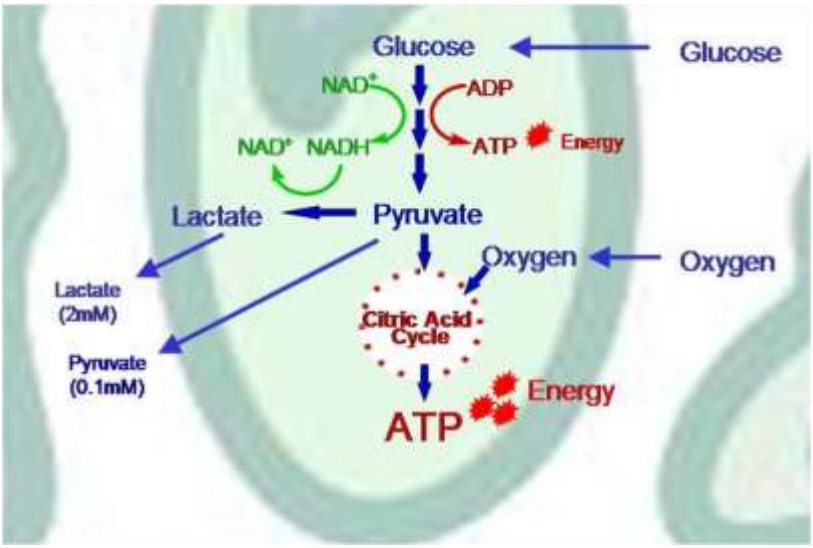

Fig 3. Glycolysis and the lactate/pyruvate ratio. The anaerobic glycolysis leads to the production of lactate and pyruvate that enters the surrounding fluid where they are taken up by the microdialysis catheter. The aerobic part of the glycolysis utilizes the citric acid cycle to produce the majority of energy in the form of ATP. This is a simplified diagram where the interplay between neurons and glia in not illustrated.

The decrease in glucose delivery from blood capillaries causes a fall in the glucose concentration in the interstitial fluid. This leads to a decreased production of pyruvate due to the lack of glucose. In the dialysate this is seen as a fall in pyruvate and a further increase in lactate and the lactate/pyruvate ratio that may occur before the onset of intracranial hypertension (Belli *et.al.* 2008). Vespa *et al.*, (2005) compared the lactate/pyruvate ratio with positron emission tomography

(PET) for metabolism of glucose and oxygen and concluded that an increase in lactate/pyruvate ratio is a sign of metabolic crises, most probably caused by mitochondrial dysfunction, that is not necessarily synonymous with ischemic cell damage. This emphasizes the importance of using glycerol as a marker of cell membrane decomposition and cell damage (see below) in addition to the lactate/pyruvate ratio.

The use of a ratio between two analytes has the advantage of abolishing the influence of changes in catheter recovery; as such a change will influence lactate and pyruvate to a similar degree. *Therefore the lactate/pyruvate ratio may be used to compare the state of different tissues in one individual as well as in different individuals.* The ratio is essentially the same in all tissues i.e. about 20. **We consider a ratio above 25 as an early warning of beginning metabolic crises.**

Lactate alone is not a good marker for ischemia as lactate may increase due to increased cell metabolism. In that case there is a parallel increase in pyruvate and the lactate/pyruvate ratio is not increased.

The normal lactate concentration in the dialysate from the brain of a sedated patient is approximately 2 mM and the pyruvate concentration 120 µM when using a 10 mm dialysis membrane and a perfusion flow of 0.3 µl/min (Reinstrup *et al*., 2000).

GLYCEROL

Glycerol is an integral component of cell membranes (Fig 4). Loss of energy due to ischemia leads to an influx of calcium into cells, activation of phospholipases and eventually to a decomposition of cell membranes, which liberates glycerol into the interstitial fluid (Hillered *et al*., 1998).

Considering the fast changes in glycerol concentration in vulnerable peri-contusional brain tissue, which are often related to changes in Cerebral Perfusion Pressure (CPP), it seems likely that cells may react by “leaking” more or less glycerol due to the severity of the ischemia.

The normal glycerol concentration in the dialysate from the brain of a sedated patient when using a 10 mm dialysis membrane and a perfusion flow of 0.3 µl/min is approximately 50-100 µM (Reinstrup *et al*., 2000).

In subcutaneous adipose tissue, on the other hand, glycerol originates from the splitting of fat (triglycerides) into free fatty acids and glycerol. This process is controlled mainly by the local sympathetic noradrenalin nerve terminals. Glycerol in subcutaneous tissue is therefore an indirect marker of sympathetic tone in the dermatome where the catheter is inserted (Hagstrom-Toft *et al.,* 1993).

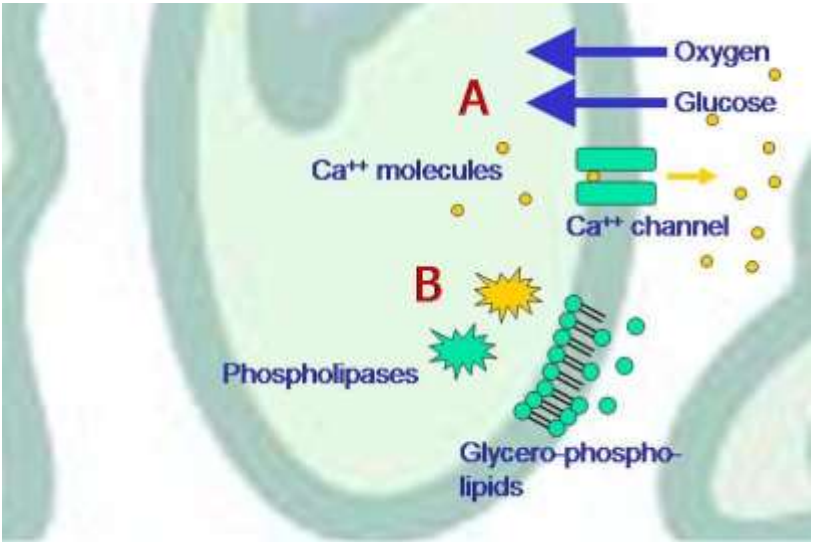

Fig 4. Glycerol and cell membrane damage. (A) If the supply of oxygen and glucose is sufficient there is enough energy to activate the calcium channels transporting calcium out of the cell. (B) In case of energy failure, calcium leaks into the cell and activates the phospholipases. Glycerol molecules are split from the fatty acids and released into the interstitial fluid where they are taken up by the microdialysis catheter as a sign of cell membrane damage.

During intensive care a subcutaneous catheter may be inserted in the peri-umbilical region to monitor glycerol as an indicator of sympathetic “stress” and glucose as an indicator of the systemic blood glucose levels (Ståhl *et al*., 2001). The normal glycerol concentration in the dialysate from subcutaneous tissue of a sedated patient when using a microdialysis catheter with a 30 mm dialysis membrane and a perfusion flow of 0.3 µl/min is approximately 200 µM. *Subcutaneous microdialysis catheters are CE labeled but not yet FDA cleared.*

GLUTAMATE

During ischemia there is an increased concentration of glutamate in the dialysate, probably due to a decrease of glutamate uptake into glial cells due to insufficient energy supply. Glutamate may open neuronal calcium channels initiating a pathological influx of calcium thus provoking cell damage. In this way an increasing level of glutamate in the dialysate from the human brain is an early, indirect marker of cell damage as well as a failing energy levels. However, it is difficult to interpret changes in brain glutamate due to the fact that glutamate release from neurons is mixed with a metabolic pool of glutamate.

The normal glutamate concentration in the dialysate from the brain of a sedated patient when using a 10 mm dialysis membrane and a perfusion flow of 0.3µl/min is approximately 10 µM and somewhat higher in a non-sedated patient (Reinstrup *et al*., 2000).

GLUCOSE

As the primary source of energy to the brain, glucose is an important marker of changes in brain metabolism. Glucose levels in the dialysate from human brain may, however, change for several reasons:

- Ischemia i.e. an insufficient capillary blood flow. Less glucose is delivered to the microdialysis catheter and the concentration in the dialysate *decreases* following a very similar pattern as brain tissue oxygen (Hlatky *et.al.* 2004).

- Hyperemia i.e. an increase in capillary blood flow. More glucose is delivered to the microdialysis catheter per time unit and the concentration in the dialysate *increases*. This happens because the recovery over the dialysis membrane is less than 100%.

- Hyperglycemia i.e.an increase in blood glucose concentration. More glucose is delivered to the microdialysis catheter and the concentration in the dialysate *increases*. This happens also if the recovery is 100%.

- Hyper metabolism or hypo metabolism i.e. an increase or decrease of glucose uptake into cells e.g. a shift from aerobic to

anaerobic metabolism. This will affect the amount of glucose in the tissue available to the microdialysis catheter causing a *decrease* or *increase* in the dialysate concentration.

If the change in brain glucose is parallel to a change in blood glucose the change is in all probability due to a variation in systemic glucose.

If the change in brain glucose is not parallel to a change in blood glucose it is probably due to changes in brain capillary perfusion or cell metabolism..

Brain glucose is of special importance today due to the increasing interest in keeping blood glucose within tight limits with the help of insulin therapy. Vespa *et.al.* (2006) found that “Intensive insulin therapy results in a net reduction in microdialysis glucose and an increase in microdialysis glutamate and lactate/pyruvate without conveying a functional outcome advantage”. Oddo *et.al.* (2008) concluded that “The use of cerebral microdialysis may provide additional protection against brain neuroglucopenia, by allowing clinicians to regulate systemic glucose levels in a way that avoids significant cerebral substrate depletion” (Fig 5).

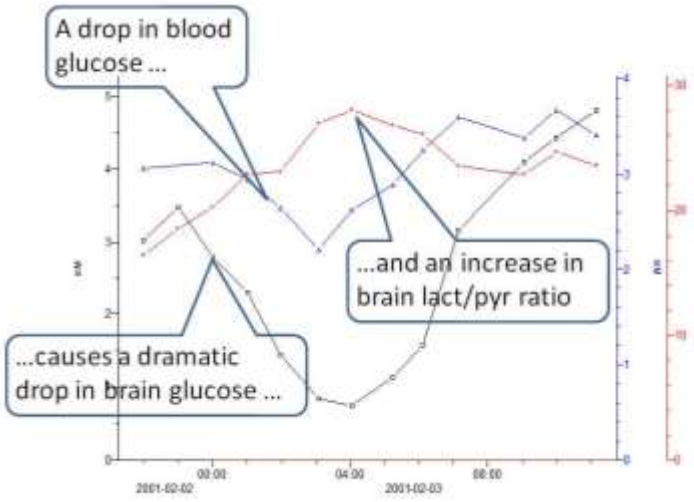

Fig 5. A drop in blood glucose in a TBI patient has a profound effect on brain glucose resulting in an increase in lactate/pyruvate ratio and brain hypoglycemia.

The normal glucose concentration in the dialysate from the brain of a sedated patient when using a 10 mm dialysis membrane and a perfusion flow of 0.3 µl/min is approximately 2 mM (Reinstrup *et al*., 2000).

POSITIONING THE MICRODIALYSIS CATHETERS

Microdialysis monitors the local chemistry of an area of the brain roughly corresponding to the length of the catheter membrane and a diameter of a few mm. The interpretation of microdialysis data therefore depends upon the position of the catheter in relation to the existing pathology. The first clinical microdialysis catheters appearing on the market were not visible on CT. Several clinical studies in the literature are therefore difficult to interpret as we do not know if the microdialysis catheters ended up in normal tissue, penumbra tissue surrounding a contusion or in dead tissue.

Catheters available today are visible on CT due to their gold tip. It is of great importance for the interpretation of bedside microdialysis data that the position of a catheter in the brain is determined from CT (Ståhl *et al*., 2003). Only then is it possible to use microdialysis data effectively to provide an early warning of secondary insults and to evaluate the result of various clinical interventions aimed at improving the condition of brain tissue during neurointensive care.

It is important to adopt a consistent strategy of where to place catheters, for example in the penumbra surrounding a mass lesion, in the region most likely to be affected by vasospasm after subarachnoid hemorrhage and/or in “normal” brain tissue.

THE CONSENSUS PAPER

The general principles of where to place catheters in different categories of patients have been agreed upon in a Consensus paper authored by experienced users of microdialysis in neurointensive care (Bellander *et al.,* 2004).

The paper states: The purpose of performing microdialysis after TBI or SAH is therefore to make possible an early detection of biochemical changes, which are early markers of tissue ischemia and may be undetected by more conventional monitoring techniques. The purpose is also to monitor the effect of therapeutic interventions aimed at preventing or alleviating ischemia in an attempt to avoid secondary insults.

The participants of the Consensus meeting recommend the following procedures when using intracerebral microdialysis in SAH and TBI:

Subarachnoid Hemorrhage

Patients: Severe cases needing monitoring of intracranial pressure and cerebral perfusion pressure.

Placement of catheters: The catheter should be placed in the tissue at risk (most likely the parent vessel territory).

Unreliable values: Due to insertion artifacts there is a period of unreliable values for at least 1 h after insertion.

Chemical markers: Glutamate and lactate/pyruvate ratio are sensitive markers for the development of ischemia. Lactate alone is insufficient as a marker of brain ischemia.

Clinical use: Microdialysis in association with other brain monitoring techniques may assist in delivery of targeted therapy for prevention of secondary ischemic injury.

Traumatic Brain Injury

Patients: Severe cases needing monitoring of intracranial pressure and cerebral perfusion pressure.

Placement of catheters: In patients with diffuse injury one catheter may be placed in the right frontal region. In patients with focal mass lesions one catheter should be placed in peri-contusional tissue. A second catheter may be placed in normal tissue. The catheter should not be placed in contusional tissue.

Unreliable values: Due to insertion artifacts there is a period of unreliable values for at least 1 h after insertion.

Chemical markers: The lactate/pyruvate ratio is a sensitive marker of brain redox state and secondary ischemic injury. Glucose, glycerol, and glutamate are additional markers of developing ischemia.

Clinical use: Microdialysis in association with other brain monitoring techniques may assist in delivery of targeted therapy for prevention of secondary ischemic injury.

IMPLANTING MICRODIALYSIS CATHETERS

In the ICU it is often convenient to place catheters through cranial bolts avoiding the need to bring the patient into the operating room. However, it is difficult to position the catheter in a select region of the brain when using bolts as there is no provision for changing the depth or angle of the catheter (Fig 6).

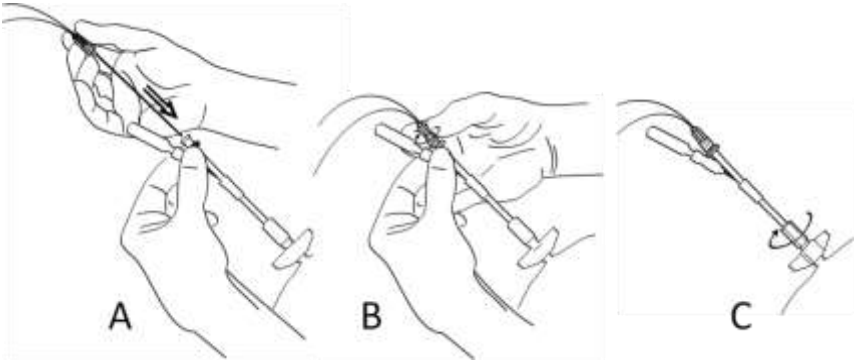

Fig 6. Implanting through a cranial bolt. A: After fixing the bolt to the skull bone the 70 Bolt Catheter is passed through the entrance tube. **B**: The luer lock connector of the catheter is fixed to the tube connector. **C:** The tube channels are sealed by tightening the compression screw.

The 70 Bolt Catheter is designed for implantation in brain tissue through the IM2 multi-lumen bolt for 2 brain probes or the IM3 multi-lumen bolt for 3 brain probes from Integra Life Sciences. The shaft of the catheter has a reinforced stainless steel section located where the compression screw of the bolt is to be tightened. If more than one catheter is entered through the bolt it is advisable to enter the microdialysis catheter before other catheters e.g. the ICP and the Brain tissue oxygen catheter.

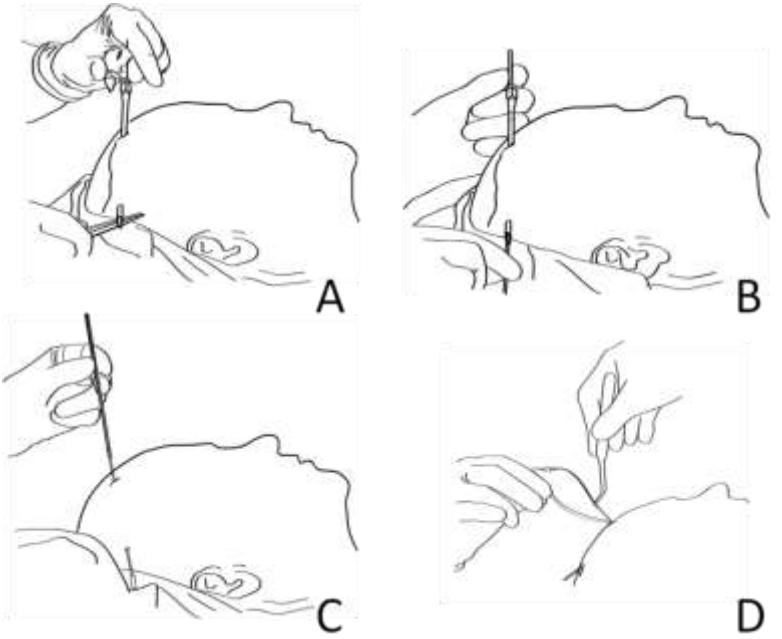

Fig 7. Percutaneous implantation A: After drilling a hole in the skull bone and opening the dura, a tunnelator is passed under the scalp. **B:** The 70 Brain Catheter is passed through the tunnelator and the tunnelator is then retracted. **C:** The protection tube is removed from the catheter by turning it counter clock wise. **D:** The catheter is held firmly at the shaft (do not touch the membrane) by a forceps or by hand and passed into the brain through the hole in the dura. The inlet and outlet tubings are stretched and the fixation cuff is sutured to the skin (see fig 7).

Due to the expense and lack of adjustable depth placement when using a bolt, catheters are often tunnelated and implanted through a burr hole making it possible to better aim for a predefined region of the brain. This is done safely and efficiently in the ICU. The percutaneous implantation procedure is described in detail by Poca *et al.,* 2006.

The tunnelation can be done in two ways: Using a tunnelator with a diameter that allows the protection tube to remain on the catheter (Fig 7) or using a thin Venflon cannula. This requires the protection tube to be removed before the catheter is entered through the tip of the cannula.

When the patient is brought into the operating room for surgery with removal of a bone flap it is easy to place the catheter under visual inspection into the peri-contusional penumbra of a lesion or in the territory of the parent vessel of an aneurysm (Fig 8). The catheter is tunnelated under the scalp and a small incision is made through the dura, subarachnoidea and pia. The dialysis membrane is positioned in the penumbra, usually 1 cm from the border of the lesion or in the region most likely to be affected by vasospasm after hemorrhage. In our own experience we have seen no consistent difference in the chemistry if the catheter is placed in white or gray matter. However, it is to be expected that the levels of neurotransmitters may vary depending upon the position.

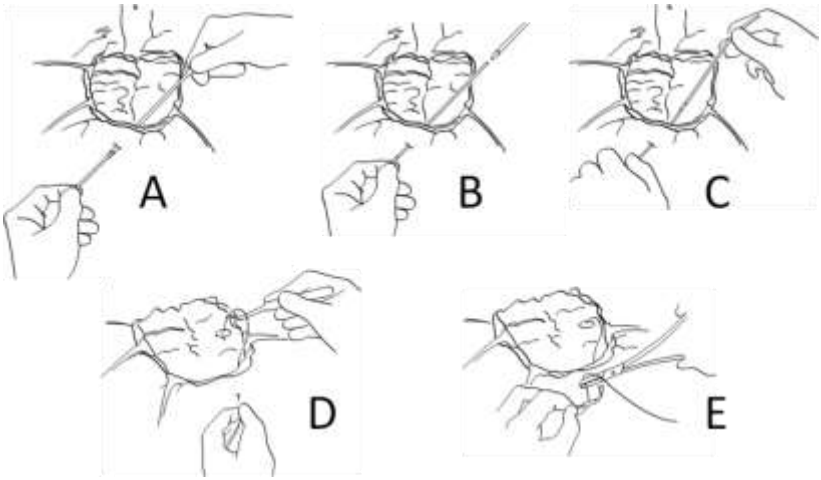

Fig 8. Implanting during open surgery. A: During open surgery the dura is repositioned. A hole is cut in the dura where the catheter will be introduced. A tunnelator is passed under the scalp. The 70 Brain Catheter is passed through the tunnelator. **B:** The tunnelator is retracted. **C:** The protection tube is removed by turning it counter clock wise. **D:** The catheter is held firmly at the shaft (do not touch the membrane) by a forceps or by hand and passed into the brain through the hole in the dura. **E:** The inlet and outlet tubings are stretched and the fixation cuff is sutured to the skin. In this way the catheter will not be pulled out if, for example, the attached microdialysis pump drops out of the patient's bed.

Regardless of how the catheter is introduced into the brain it is important to locate the gold tip of the catheter on the first CT performed after implantation (Fig 9). The location of the catheter will determine how relevant the biochemical data are for the interpretation of brain pathology and the early warning of a secondary insult (Engström *et.al.* 2005).

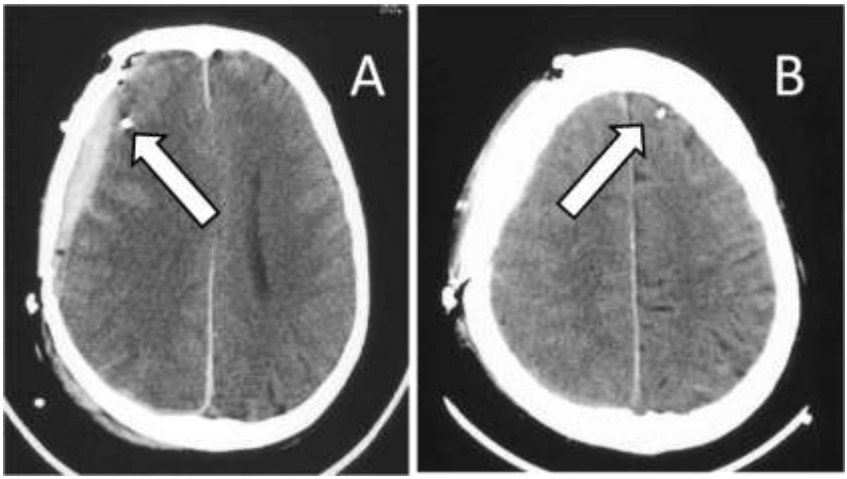

Fig 9. Locating the tip of the microdialysis catheter. A: Epidural hematoma. The arrow points at the gold tip, which is easily seen on the CT. **B:** Contralateral side with visible gold tip.

CONNECTING THE MICRODIALYSIS PUMP

Once the microdialysis catheter is implanted and the wound closed the nursing staff fills the microdialysis syringe with 2.5 ml of artificial CSF, connects the syringe to the catheter inlet tube and places a microvial in the vial holder of the catheter outlet tube. The syringe is then placed in the microdialysis pump. As soon as the lid is closed a flush sequence starts, which removes all air from the tubing and catheter. The flush flow is 15µl/min and lasts for 6 min. The pump then changes its flow to 0.3 µl/min. Blinking diodes indicate the stages in the pump sequence.

If the catheter is implanted in the operating room it is advisable to wait with the connection of the catheter to the syringe and pump until the patient is in the ICU.

If no fluid appears in the microvial the flush sequence may be repeated by opening and closing the lid of the pump. This may also be done anytime during microdialysis monitoring if the flow stops as indicated by empty vials. However, a flush sequence dilutes the sample and the concentration of the biochemical markers will be lower. It is important to make a note about the flush in the patient protocol to avoid misinterpretations of the low levels of biochemical markers.

Fig 10. The 106 MD pump is a small battery driven pump with a flow of 0.3 µl/min. The pump is loaded with a syringe filled with 2.5 ml of artificial CSF and connected to the luer connector of the 70 Brain microdialysis catheter. Using 0.3 µl/min flow, 2.5 ml is sufficient for 5 days of microdialysis. The 107 MD pump allows the adjustment of flow between 0.1 - 5 µl/min.

PERFUSION FLOW

The flow of the standard microdialysis pump (Fig 10) is 0.3 µl/min, which gives approximately 70% recovery when using a 10 mm dialysis membrane (Hutchinson *et al.,* 2000 and 2002). Catheters with 20 or 30 mm membrane will give next to 100% recovery in the brain. There may be situations when it is necessary to use a higher perfusion flow, for example when sampling frequently and the 0.3 µl/min flow does not give enough sample volume to permit analysis. This may be the case during intra-operative microdialysis when samples are changed every minute in order to monitor ischemia during e.g. temporary clipping.

The 107 MD pump allows adjustment of the flow between 0.1 – 5 µl/min. A flow of 1µl/min will give a recovery of approximately 30% using a 10 mm membrane (Hutchinson *et al*., 2002). When the intention is to determine the true interstitial concentration of a compound in the tissue the flow can be stepwise decreased down to 0.1 µl/min in order to find a flow where the concentration is stable i.e. a flow that gives 100% recovery (The 107MD pump is not yet FDA approved and must be used only after IRB approval).

Another reason to use a high perfusion flow is to reduce the time delay occurring when the dialysate flows from the brain to the microvial. The delay is in the range of 20 min when the perfusion flow is 0.3 µl/min but can be reduced to close to 1 min if the flow is increased to 5 µl/min. However, during neurointensive care chemical changes usually take place over several hours and a 20 min delay is of minimal consequence.

During normal intensive care it is highly advisable to use the standard flow and standard catheter as this will make it possible to compare data over time between patients in the same or different clinical departments.

POINT OF CARE ANALYSIS

The extremely small sample volumes produced during microdialysis monitoring is equivalent to one droplet/hour of a fluid with a low concentration of biochemical markers. This requires special equipment able to handle unusually small volumes. This includes catheters, microvials, pumps, and a very sensitive biochemical analyzer capable of displaying brain chemistry as trend curves during the entire treatment period in the ICU.

The ISCUSflex Microdialysis Analyzer displays trend curves of the different analytes as well as the range of normal values for each analyte on an integral pressure sensitive screen (Fig 11). The last recorded value is displayed numerically and “indicator arrows” show the trend calculated over several hours. The instrument can display data from 8 patients simultaneously and allows batch analysis of up to 16 samples off line.

Fig 11. The ISCUSflex© Microdialysis Analyzer can monitor up to 8 patients simultaneously, and a total of 16 catheters. There are reagents for Glucose, Lactate, Pyruvate, Glycerol, Glutamate and Urea. A removable vial cassette with 16 positions for batch analysis makes it possible to analyze samples “off line”. Calibration to an internal standard is done automatically every 6 hrs. There are excellent possibilities to transfer microdialysis data to an external computer via USB, SD card or a network cable.

A MICRODIALYSIS ALGORITHM

The following is an “algorithm” describing how microdialysis is performed in our clinic. The text applies to the ISCUSflex Microdialysis Analyzer, which accepts up to 8 patients and 5 reagents as well as the CMA600 analyzer for 3 patients and 4 reagents:

1. Patient arrives in the emergency room

2. Starting the analyzer

- Start up the analyzer in the ICU
- Load Rinsing Fluid
- Prepare and load reagents

3. Implanting the Brain catheter(s):

Alternative 1:

- Patient arrives in the ICU
- Implant brain catheter: Percutaneous or Bolt

Alternative 2:

- Patient arrives in the Operating room
- Implant brain catheter: Percutaneous, Bolt or open surgery
- Patient is transferred to the ICU

4. Starting Microdialysis sampling:

- Fill the microdialysis syringe (not more than 2.5 ml; no air bubbles)
- Connect the syringe to the catheter
- Place the syringe in the pump (tip first, piston second (it is advisable to change the battery for every new patient)
- Start the perfusion by closing the lid of the pump (for safety tape down the lid; use tape to write down start time and date)
- The flush cycle starts in order to remove air in the tubings (15 µl/min for 6 min, fast blinking diodes)
- The flow changes to 0.3 µl/min (106 MD pump) or the preset flow (107 MD pump) (slow blinking diodes)
- Find a secure place for the pump making sure that it does not fall out of the bed or end up under the patient.
- Place a microvial in the vial holder of the catheter
- After a while return to make sure that fluid is collected in the vial
- Discard the first vial after 30-60 min (the sample is diluted because of the flush).
- Place a new vial in the vial holder

5. Analyzing Microdialysis samples:

- Register the patient in the analyzer if you have not done it yet
- Check that the analyzer calibration is OK
- Change vials every 60 min (shorter interval if the clinical condition gets worse; you can change every 10 min if necessary)
- Remove the vial containing new dialysate from the vial holder of the catheter
- Place a new vial in the vial holder

Alternative 1:

- Remove and discard the vial in the analyzer
- Place the vial containing the new dialysate in the analyzer

Alternative 2:

- Remove the vial in the analyzer and place it in a Vial Rack, which is kept in a fridge. In this case vials must be prelabeled with an identification number.
- Place the vial containing the new dialysate in the analyzer
- Once the vial rack is filled (12 vials) the rack is transferred to a household freezer.
- Samples can be stored for a limited period of time (depending upon the compound of interest) and re-analyzed e.g. by HPLC, Capillary electrophoreses, Mass Spectrometry etc. NOTE: After thawing vials have to be briefly centrifuged to remove air bubbles (see separate instructions from M Dialysis)

Alternative 3:

- The vials are not analyzed bedside
- Remove samples from the vial holders and place them in a Vial Rack kept in a fridge. In this case vials must be prelabeled with an identification number.
- Once the vial rack is filled (12 vials) the rack is transferred to a household freezer.
- Samples can be stored for a limited period of time (depending upon the compound of interest) and then analyzed as batches of 24 samples at a time in the CMA600 and 16 at a time in the ISCUSflex Microdialysis Analyzer. NOTE: After thawing vials have to be briefly centrifuged to remove air bubbles (see separate instructions from M Dialysis)

6. Monitoring Brain chemistry:

- A post implantation CT is of great value in order to determine the position of the catheter in relation to the tissue pathology and in this way evaluate the relevance of tissue chemistry data
- Check the screen of the analyzer every hour using the ”LTC method” i.e. observing **L**evel, **T**rend and **C**ompare to e.g. ICP, CPP and tissue oxygen

- Alert the MD on duty if the chemical condition of the tissue changes, especially when levels and trends are out of normal range
- Evaluate therapeutic interventions, e.g. changes in CPP, blood glucose, oxygen saturation etc, against changes in tissue chemistry over time
- Microdialysis data are typically printed every day by the internal printer and the printout is attached to the patient journal
- The overall intention is to restore tissue chemistry to normal by ***individualizing*** the treatment of the patient

7. Storing patient data:

- The CMA600 stores patient data permanently on the hard drive. Once the patient leaves the ICU microdialysis data can be printed and added to the patient record and/or transferred electronically to an external database.
- The ISCUSflex Microdialysis Analyzer stores patient data for 6 weeks. It is important that data are transferred to an external computer as soon as the patient leaves the ICU, e.g. by a USB memory. Using the LAB Pilot software (M Dialysis) data can be permanently stored, printed for the patient record and analyzed in relation to other patient data in the database.
- The interpretation and safety of Microdialysis data is greatly improved if the ISCUSflex Microdialysis Analyzer is connected on line, by a network cable, to a computer using the ICU Pilot software, i.e. an extended version of the LAB Pilot software.
- ICU Pilot offers the additional possibility to connect the computer to other instruments around the patient such as the ICU monitor, ventilator, Licox oxygen monitor (Integra Life Sciences) etc. and in this way integrate microdialysis data in a multimodal monitoring system. All recorded variables can be dynamically compared with each other and with historical data from patients with similar pathology and in this way predict outcome and decide about clinical interventions.
- ICU Pilot SQL offers the most elegant solution when all patient data can be read from a central patient data base and dynamically displayed in graphic form bedside in order to achieve true multimodal monitoring capability

8. Trouble shooting - no fluid in the microvial

- Check the pump flashing diodes for malfunction messages.
- Is the battery low? The lid open? The luer connection leaking? The syringe empty? The catheter tubing kinked?
- If no cause is found: Open and close the lid of the pump to initiate a flush. This will fill up the system and expel any air. Examine the vial for liquid. Repeat two or three times if necessary. Make a note in the protocol to explain the sudden decrease in dialysate concentration due to the dilution caused by the flush.
- If still no fluid in the vial call the M Dialysis support hot line.

Fig 12. Multimodal monitoring allows for the display of all data as trend curves on one computer screen. It creates the framework for *individualizing* therapy on the basis of clinical status, brain tissue chemistry and the effect of therapeutic interventions.

MULTIMODAL MONITORING

In order to make effective use of microdialysis data it is essential to relate them to other data collected bedside e.g. ICP, CPP or brain tissue oxygen (Fig 12). This can be done with the ICU Pilot© software, which runs on the PC controlling the CMA 600 or on an external PC connected to ISCUSflex Microdialysis Analyzer (Fig 13). The ICU monitor etc are connected to the PC by serial cables. Another possibility is to use the ICU Pilot SQL software, which can read all patient data stored in an SQL

database. The ICU Pilot software allows powerful data manipulation in order to highlight important changes in the status of the patient as well as the effect of various therapeutic interventions. After the patient is discharged from the ICU, data can be stored in a database containing the patient record.

The ICU Pilot screen gives instant access to all “multimodal” information. It can be configured to show any relationship between biochemical and physiological variables in the form of user defined templates. Variables can be compared with each other instantaneously by simple drag and drop.

The information is presented as trend curves on the screen and continuously updated as ICU pilot collects data from the ICU monitor. The trend curves make it possible to evaluate the gradual improvement or deterioration in the patient's condition. It is possible to move to any previous time point in order to examine the effect of previous therapeutic interventions or changes in the condition of the patient.

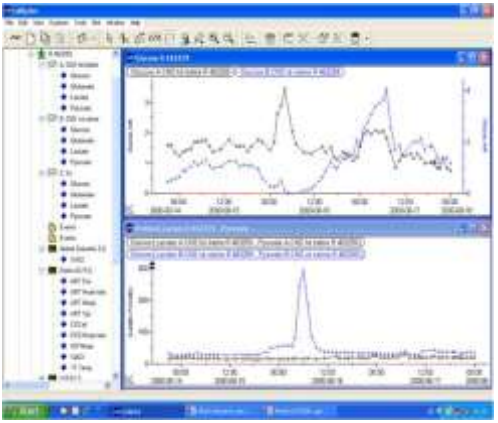

Fig 13. ICU Pilot screen from the PC connected to the ISCUSflex Microdialysis Analyzer. All recorded variables are displayed in the left column and can be compared to each other by simple drag and drop or by being included in user defined template windows.

Data from other patients or from statistical averages of large groups of patients can be dragged and dropped into the graphs of the patient

under treatment in order to compare and predict outcome (Fig 14 & 15).

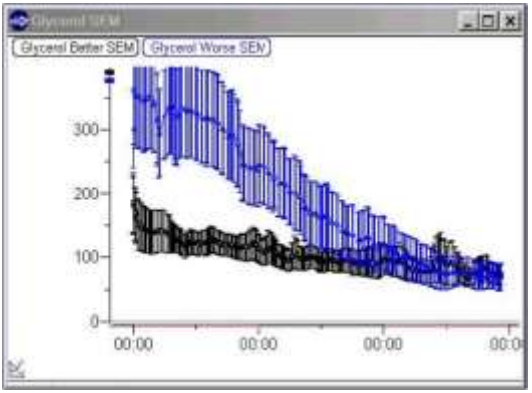

Fig 14. Reference values. Glycerol in the penumbra (blue) is initially high but after 2 1/2 days it has reached the same level as in the normal brain (black).

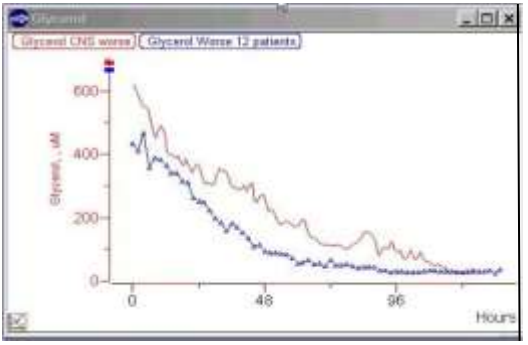

Fig 15. Outcome prediction. The glycerol levels from this patient (red) are high and stay well above the reference values (blue) during five days. It reaches normal levels two days after the reference group (12 patients), which is an indication of worse outcome than the average TBI patient.

Separate windows with user defined “templates” highlights important relationships. These windows will be the same from patient to patient making it easy for the staff to recognize and evaluate the information.

Typical use of template windows is to get an in depth understanding of how pressure affects cell ischemia by including ICP, CPP and the

lactate/pyruvate ratio in the same graph.

INTERPRETING MICRODIALYSIS DATA

During intensive care brain chemistry often changes profoundly in the patient. At our present state of knowledge it is impossible to interpret every change, however, major pathological states manifest themselves as dramatic increases or decreases of the chemical markers.

The first hours of microdialysis data give an indication of how severely brain tissue is affected in the peri-contusional penumbra. This information gives a reference value for determining if tissue physiology is improving or deteriorating.

The implantation of a microdialysis catheter inflicts a certain amount of trauma to the brain tissue. This is well known from animal studies and it usually takes an hour or more before baseline values are reached after an implantation. In the human brain this is particularly evident for glutamate and sometimes for glycerol. However, in a clinical setting the time between implantation of the catheter and the actual use of microdialysis data is often longer than an hour due to all procedures taking place around the patient.

The range from normal to pathological levels of different analytes are well known from normal brain tissue in patients with posterior fossa tumors (Reinstrup *et al*., 2000) and from damaged as well as “normal” brain tissue in TBI and SAH patients. Normal levels differ strongly from pathological levels e.g. in severe brain trauma (see below).

THE LTC-METHOD OF DATA INTERPRETATION

It is necessary to apply a simple and straight forward method when evaluating multimodal data during neurointensive care. The LTC-method (**L**evel, **T**rend, **C**omparison) represents a systematic way of looking at microdialysis data alone and in comparison with other data displayed by the ICU pilot software. By going through this simple routine it is possible to get a quick grasp of the patient’s condition.

1. **Level**: Are the levels of microdialysis markers within the physiological range? This range is shown on the screen of the Analyzer as a colored band in the graph.

2. **Trend**: Is microdialysis chemistry becoming more or less pathological over time as displayed by the curves on the ISCUSflex or ICU pilot screen?

3. **Comparison**: How does microdialysis chemistry compare with other recorded variables, for example ICP and CPP? Use ICU pilot to display different data in the same graph.

SUBARACHNOID HEMORRHAGE

Microdialysis has been used extensively for monitoring ischemia in SAH patients (Fig 16). Nilsson *et al*., (1999) described the detailed biochemistry of vasospasm and concluded that lactate and glutamate may be the most sensitive and early markers for incipient ischemia followed by the lactate/pyruvate ratio and glycerol during manifest ischemia and cell degeneration. They found that metabolic changes preceded the increase in blood flow velocity as recorded by TCD (Trans Cranial Doppler)

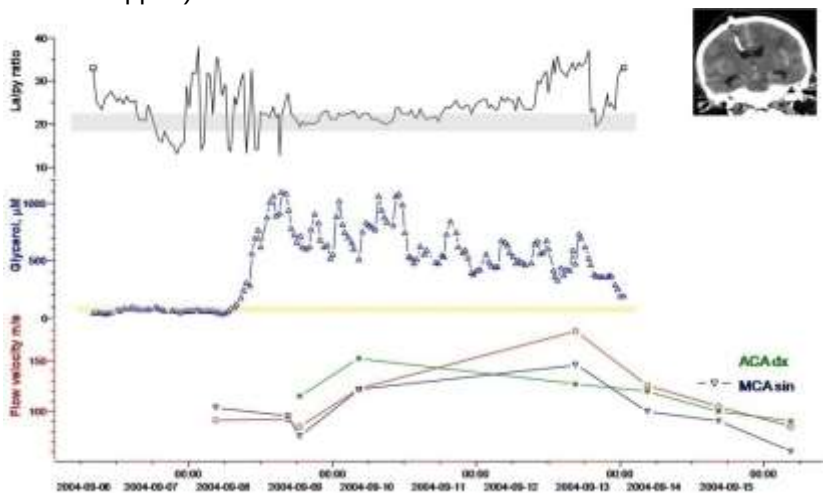

Fig 16. Vasospasm after SAH. The decreasing lactate/pyruvate ratio suddenly increases far above normal levels as an early warning of beginning ischemia. The dramatic increase in glycerol indicates brain cell damage. The Trans Cranial Doppler (TCD) reading shows spasm much later than the biochemical markers. CT shows the position of the intraventricular drain and the gold tip of the microdialysis catheter.

Sakowitz *et al*., (2001) concluded that microdialysis “can be carried out routinely in the ICU-setting to detect and monitor patterns of metabolic impairment. Compared to TCD it has a remarkable specificity making it a well-suited method to monitor delayed ischemic neurological deficits following aneurysmal haemorrhage”. Skjøth-Rasmussen *et al*., (2004) found that the ischemic pattern after SAH preceded the occurrence of delayed ischemic neurological deficits by a mean interval of 11 hours.

TRAUMATIC BRAIN INJURY

In 1995 CE-labelled microdialysis catheters intended for human use and an instrument for bedside analysis of glucose, lactate, pyruvate, glycerol and glutamate became available on the market from CMA Microdialysis, Stockholm. This enabled us to start routine monitoring of patients with TBI and SAH in Lund. Today the patient data base in Lund comprises close to 400 patients. In our first report on normal brain we established baseline values for the energy related metabolites (Reinstrup *et al*., 2000).

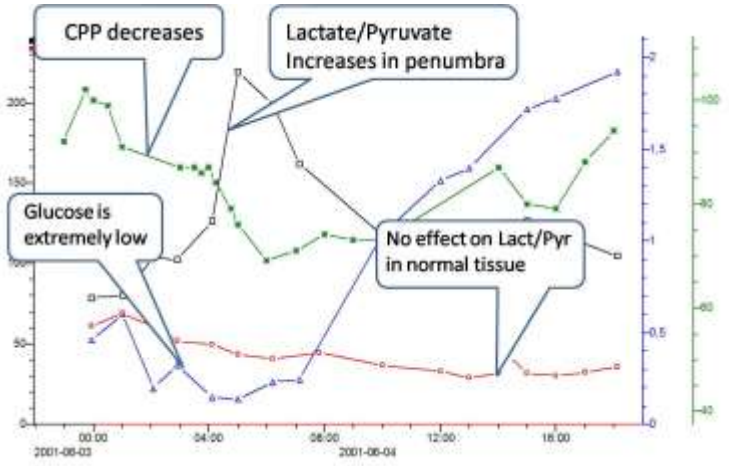

Fig 17. Ischemic episode. CPP decreases and lactate/pyruvate ratio increases and glucose decreases due to insufficient capillary perfusion.

In view of previous findings we placed one catheter in the peri-contusional penumbra tissue and a second catheter in normal tissue, usually through a second burr hole in front of the intraventricular ICP catheter. We found that microdialysis can be performed on a routine

basis by the regular staff in an ICU and that data can be used for detecting global as well as local complications (Ståhl *et al*., 2001) (Fig 17 & 18).

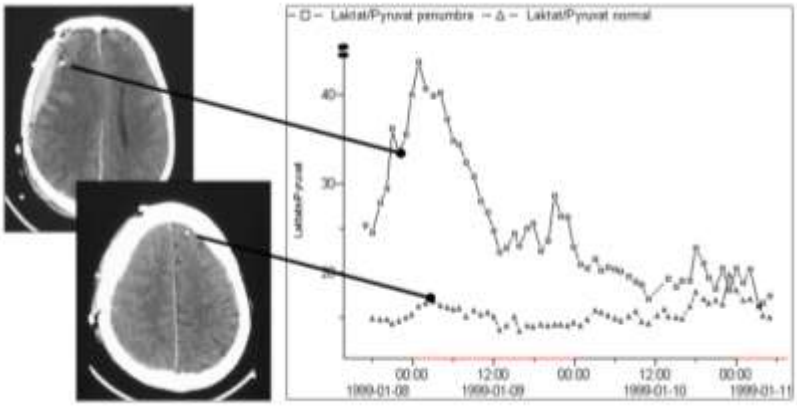

Fig18. Secondary insult in the penumbra. The gold tip of the microdialysis catheter is visible on CT and shows that one catheter is positioned in the penumbra of the hematoma and the other in normal tissue contralateral to the lesion. The dramatic rise in lactate/pyruvate ratio was due to and inadequate CPP. This was corrected by a blood transfusion which caused the lactate/pyruvate ratio to decrease and finally reach the normal level of the contralateral side. The data shows the great difference in vulnerability between penumbra and normal tissue.

Our most important observations were:

- The metabolites measured give information that is of direct clinical importance regarding substrate availability (glucose), redox state of the tissue (lactate/pyruvate ratio), degradation of glycerophospholipids in cell membranes (glycerol) and extracellular concentration of excitatory amino acids (glutamate).

- There was a great difference in the energy metabolism of the peri-contusional tissue as compared to normal tissue in the same patients.

- The biochemical consequences of severe anaemic hypoxia were observed several hours before the deterioration was detected by conventional methods (ICP & CPP).

PREDICTING OUTCOME

Microdialysis helps to predict patient outcome. This is particularly evident when comparing the mean levels of the various markers in the peri-contusional area of patients with fatal traumatic lesion (Ståhl *et al*., 2001) and values obtained during wakefulness in normal human brain (Reinstrup *et al.*, 2000). Under "Sedated" (see below) I have included the values we consider "normal" in sedated neurointensive care patients.

Analyte	**Fatal**	**Wakefulness**	**Sedated**
Glucose	0.1 mM	1.7 mM	2mM
Lactate	8.9 mM	2.9 mM	2mM
Pyruvate	31 µM	166 µM	120µM
Lactate/Pyruvate	458	23	15-20
Glycerol	573 µM	82 µM	20-50µM
Glutamate	381 µM	16 µM	10µM

Vespa *et.al.* (2003) found that "...the level of extracellular glucose is typically reduced after traumatic brain injury and associated with poor outcome..." and in a recent study Oddo et.al. (2008) reported that tight systemic glucose control is associated with reduced extracellular levels of glucose in the brain, which in turn correlated with increased mortality. Marcoux et.al. (2008) found that elevated lactate/pyruvate ratio was associated with increased frontal lobe atrophy at 6 months after TBI. Sarrafzadeh et.al. (2004) concluded that "The L/P ratio was the best metabolic independent prognostic marker of 12-month outcome" after SAH.

In a study using bedside microdialysis after large human MCA infarctions Berger et.al. (2008) concluded that "Rescue of peri-infarct tissue and/or prevention of secondary ischemic injury could be associated with a lower mortality in invasively treated patients".

BIBLIOGRAPHY OF BRAIN MICRODIALYSIS

The following is a short selection of papers describing biochemical findings of particular relevance for the early detection of secondary damage and the evaluation of therapeutic interventions during neurointensive care.

Microdialysis of the human brain was first performed in 1987 at the Karolinska institute in a Parkinson patient subject to thalamic lesion for alleviating tremor (Meyerson *et.al.* 1990).The catheter was introduced stereotaxically and samples were collected every 10 min and analyzed for a large number of neurotransmitters and metabolites. We found that baseline levels of the various analytes in the dialysate were much higher than in animals due to the possibility of using a much larger dialysis membrane. Even more important, baseline levels were reached much faster probably due to the small implantation trauma due to the large size of the human brain.

We then performed the first study on brain ischemia in Uppsala monitoring the brain chemistry in tissue, which was resected during tumor surgery (Hillered *et.al*. 1990). This led to a study of microdialysis during neurointensive care of TBI and SAH describing changes in especially lactate, pyruvate and glutamate (Persson and Hillered, 1992).

In cooperation with neurosurgery in Lund and CMA Microdialysis AB we then develop flexible catheters more suitable for implantation in human brain and a microdialysis analyzer designed for bedside use.

Subarachnoid Hemorrhage

Microdialysis has been used extensively for monitoring ischemia in SAH patients. Säveland *et.al.* (1996) found that increased levels of glutamate correlate well with clinical course and neurological symptoms after SAH.

Persson *et.al.* (1996) found an increase in glutamate when the lactate/pyruvate ratio reached values of approximately 25 or above. Lactate/pyruvate ratio appeared to be a more reliable marker compared to lactate alone and there was a statistically significant correlation

between lactate/pyruvate ratio and clinical outcome during day 0-4, which did not exist for lactate.

Nilsson *et.al.* (1999) described the detailed biochemistry of vasospasm and concluded that lactate and glutamate may be the most sensitive and early markers for incipient ischemia followed by the lactate/pyruvate ratio and glycerol during manifest ischemia and cell degeneration. They found that metabolic changes preceded the increase in blood flow velocity as recorded by Trans Cranial Doppler (TCD).

In a study combining microdialysis and Positron Emission Tomography (PET) Enblad *et.al.* (1996) concluded that the energy related metabolites such as lactate and lactate/pyruvate ratio may be used as extracellular markers of ischemia.

In a series of studies Unterberg and co-workers placed microdialysis catheters 25 to 35 mm into the parenchyma of the vascular territory most likely to be affected by vasospasm (Unterberg *et.al*. 2001). Sakowitz *et.al.* (2001) concluded that microdialysis “can be carried out routinely in the ICU-setting to detect and monitor patterns of metabolic impairment. Compared to TCD it has a remarkable specificity making it a well-suited method to monitor delayed ischemic neurological deficits following aneurysmal haemorrhage”.

Sarrafzadeh *et.al.* (2002) found that lactate and glutamate are early markers of clinical vasospasm followed by lactate/pyruvate ratio and glycerol during manifest vasospasm in patients with SAH. She stated that bedside cerebral microdialysis is a safe technique for the indication of acute and delayed ischemic neurological deficits in SAH patients when inserted into the region of interest and suggests that “early detection of metabolic changes might also allow optimization of standard intensive care treatments, such as triple-H therapy”.

Skjøth-Rasmussen *et.al.* (2004) found that the ischemic pattern after SAH preceded the occurrence of delayed ischemic neurological deficits by a mean interval of 11 hours. Sarrafzadeh *et.al.* (2004) concluded that “Microdialysis parameters reflected the severity of SAH. The L/P ratio was the best metabolic independent prognostic marker of 12-month

outcome". In a recent article Nagel *et.al.* (2009) argues that "metabolism-guided, optimized ICP therapy could help minimize secondary brain damage and improve prognosis in patients with SAH".

In two recent studies Zetterling *et.al.* (2010 & 2011) found an interesting correlation between global ischemia after spontaneous SAH and significantly elevated lactate and pyruvate levels 70 to 79 hours after SAH. They suggest that this is a sign of cerebral hypermetabolism meeting the increased energy demand in the recovery phase after SAH. The evidence of a transition to a hyperglycolytic state was further supported by a gradual decline in brain glucose and the brain/plasma glucose ratio and an increase in brain pyruvate and lactate concentrations during the 1st week after SAH. In agreement with these findings Oddo *et.al.* (2011) found that a pattern of increased cerebral hyperglycolytic lactate was associated with good long-term recovery. They suggested that lactate may be used as an aerobic substrate by the injured human brain.

Traumatic Brain Injury

Persson and Hillered (1992) made the first microdialysis studies of the human brain after traumatic brain injury. They found that microdialysis can be used for long term studies of energy related metabolites and amino acids (e.g. glutamate), and that the fluctuation of these substances corresponded to various clinical events "presumably involving hypoxia/ischemia". They used the lactate/pyruvate ratio as a marker for energy disturbance in the brain.

They presented several arguments for the reliability of the lactate/pyruvate ratio. (1) Due to the structural similarity of lactate and pyruvate any change in the in vivo diffusion conditions during a pathological state could be expected to affect both metabolites similarly. (2) Being a ratio it is independent of the characteristics of the microdialysis catheter. (3) On the basis of a review of 13 papers in the literature describing the normal brain lactate/pyruvate ratio in different species they concluded that the basal level of the lactate/pyruvate ratio is below 20. This fits with the basal lactate/pyruvate ratio of 23 that we

found in normal brains of patients operated for posterior fossa tumours (Reinstrup *et.al.* 2000).

Bullock, Zauner and co-workers made the important observation that when placing the microdialysis catheter next to a cerebral contusion sustained cerebral blood flow reductions caused massive release of excitatory amino acids while in patients without secondary ischemic complications or focal contusions post traumatic glutamate release appears to be only transient (Zauner *et.al.* 1996). They conclude that sustained high ICP and poor outcome were significantly correlated to high levels of glutamate (>20 µM) (Bullock *et.al.*1998).

In 1995 CE-labelled microdialysis instruments became available (CMA Microdialysis, Stockholm). This enabled us to start routine monitoring of all patients with severe head injuries in Lund. In our first report on normal brain we established baseline values for the energy related metabolites (Reinstrup *et.al.* 2000).

We placed one catheter in the peri-contusional penumbra and a second catheter in normal tissue, usually through a second burr hole in front of the intraventricular ICP catheter. Our most important observations were:
(1) The metabolites measured give information that is of direct clinical importance regarding substrate availability (glucose), redox state of the tissue (lactate/pyruvate ratio), degradation of glycerophospholipids in cell membranes (glycerol) and extracellular concentration of excitatory amino acids (glutamate).
(2) There was a great difference in the energy metabolism of the peri-contusional tissue as compared to normal tissue in the same patients.
(3) The biochemical consequences of severe anaemic hypoxia were observed several hours before the deterioration was detected by conventional methods (ICP-CPP).
(4) We were able to compare the mean levels of the various markers in the peri-contusional area of patient with fatal traumatic lesion (Ståhl *et.al.* 2001) with values obtained during wakefulness in normal human brain (Reinstrup *et.al.* 2000).

In a study of 27 patients, treated according to the Lund concept, we documented the brain chemistry in patients with favourable outcome (Ståhl *et.al.* 2001) in contrast to the previous study of fatal outcome.

The introduction of microdialysis catheters with a gold tip visible on CT marked a quantum leap in the use of microdialysis in routine monitoring during neurointensive care. It became possible to visualize the position of the catheters in relation to the contusion or hemorrhage and thereby determine the relevance of the microdialysis data.

In our first study where the catheter position was verified we received further proof of the great difference in sensitivity to secondary insults between normal brain and the tissue of the peri-contusional penumbra (Ståhl *et.al.* 2003). In a follow up study on catheter location we concluded that “Data obtained from intracerebral microdialysis can be correctly interpreted only if the locations of the catheters, as they relate to focal brain lesions, are visualized”. And a “biochemical penumbra zone” surrounds focal traumatic brain lesions” Engström *et.al.* (2005).

In a methodological study Hutchinson *et.al.* (2000) showed that adjacent brain catheters produced equivalent results and that the recovery of a catheter with 10 mm membrane and a flow of 0.3 µl/min was approximately 70%. In a comparison of catheters with 20kDa and 100kDa cut off they found equivalent results and concluded the 100kDa catheters can, therefore, be used for routine clinical monitoring of extracellular substances, as well as research on larger molecular weight protein sampling.

The relation between microdialysis biochemical markers and patient outcome has been convincingly documented in several studies. In a large study of 223 patients Timofeev *et.al.* (2011) showed that during the initial 72 h of monitoring, median glycerol levels were higher in the mortality group and median lactate/pyruvate ratio and lactate levels were significantly lower in patients with favourable outcome. In a study of 165 patients Chamoun *et.al.* (2010) showed that patients where the glutamate levels tended to normalize during the 120 h monitoring period had a lower mortality rate and a better 6-month functional outcome than

patients where glutamate tended to increase with time or remain abnormally elevated.

Several papers document the relationship between microdialysis data and physiological parameters such as ICP and CPP where an increasing CPP coincides with a decrease in lactate/pyruvate ratio (De Fazio *et.al.* 2011). However, Vespa *et.al*. (2007) reported that while sustained increases in lactate/pyruvate ratio occurred more frequently in peri-contusional tissue compared with normal brain tissue the lactate/pyruvate ratio was not related to cerebral perfusion pressure. Lactate/pyruvate ratio values appeared to be elevated despite cerebral perfusion pressure values customarily considered to be adequate.

The explanation to the different findings related to the lactate/pyruvate ratio is probably due to that an increase in the ratio sometimes represents ischemia, which may be corrected by increasing CPP, while in other cases the ischemia has developed into mitochondrial dysfunction (Vespa *et.al*.2005), which is not alleviated by increasing CPP alone.

Middle Cerebral Artery Infarction

Several papers have demonstrated the predictive value of microdialysis in stroke patients.

Severe hemispheric stroke carry a high mortality because of the formation of fatal brain edema. Berger *et.al.* (1999) reported a case of fatal MCA (Middle Cerebral Artery) infarction were the neurochemical alterations contralateral to the infarction preceded clinical signs of herniation for several hours.

Schneweis *et.al.* (2001) used ICP and microdialysis in the ipsilateral frontal lobe in an attempt to identify MCA patients at risk and decide on invasive therapies such as decompressive hemicraniectomy or hypothermia. They found that chemical changes varied in accordance with clinical course, size of infarction and brain edema. Stable ICP and chemistry was found in patients without progressive space-occupying infarcts while increase in ICP, glutamate and lactate/pyruvate ratio was followed by massive edema and large infarcts.

Berger *et.al.* (2002) assessed the effect of therapeutic moderate hypothermia with microdialysis and were able to characterize three different brain regions with different reaction to hypothermia: (1) Non-infarcted tissue with stable chemistry and a moderate lowering of glutamate, lactate and pyruvate during hypothermia. (2) Peri-infarct tissue where hypothermia caused a pronounced lowering of glutamate, glycerol, lactate and pyruvate. (3) Irreversibly damaged tissue with excessive increases of glutamate, glycerol and lactate and lowering of pyruvate. They conclude that microdialysis is a safe and feasible method for neurochemical monitoring indicating normal brain tissue, salvageable tissue and irreversibly damaged tissue and the effect of hypothermia on these different compartments. They conclude that, future treatment strategies for life-threatening stroke should be guided by close neurochemical monitoring.

In a recent article Berger *et.al.* (2008) conclude that "Microdialysis allows bed-side monitoring of neuroprotective effects of stroke rescue therapies such as hypothermia and hemicraniectomy. Rescue of peri-infarct tissue and/or prevention of secondary ischemic injury could be associated with a lower mortality in invasively treated patients".

Nielsen *et.al.* (2012) studied 44 patients with malignant MCA infarcts after decision to perform decompressive hemicraniectomy. They found that normal interstitial glucose level in the infarcted hemisphere in combination with substantial MCA blood-flow velocities bilaterally, even before decompressive hemicraniectomy was performed, indicates that malignant brain swelling usually commences when the embolus/thrombosis has been largely resolved and recirculation of the infarcted area has started.

Implementation of cerebral microdialysis at community hospitals

The use of advanced neuromonitoring techniques are normally implemented in university hospitals before being introduced into routine clinical use in community based hospitals. It is therefore of great interest to read a recent paper from Chen e*t.al.* (2012) describing a 5-year,

single-institutional experience of cerebral microdialysis at a community-based hospital, Legacy Emanuel Medical Center in Portland, Oregon. During this period 248 cerebral microdialysis catheters were placed in 174 patients.

Patients undergoing open craniotomy for traumatic brain injury, cerebrovascular accident, aneurysmal subarachnoid hemorrhage or brain tumor resection were candidates, as well as patients with diabetes mellitus or impaired glucose metabolism to allow for better control of cerebral glucose. Catheters were implanted during open surgery or via bolt or burr hole placement. No cerebral hemorrhages or infections were attributed to cerebral microdialysis.

Only attending, board-certified neurosurgeons placed the microdialysis catheters. Nursing staff maintained and demonstrated competencies in microdialysis by completing practical and written tests. Catheter placement was reviewed with particular attention as to whether the catheter tip was in normal, edematous, or injured/hemorrhagic brain. The MD studies were not done in a dedicated neuroscience ICU, but rather in a 10-bed shared trauma, surgical, and medical ICU.

The paper states that they found microdialysis data “extremely useful in clinical decision making” as a component of multimodal brain monitoring. However, this varied on a case by case basis and was dependent on variables such as the pathology, the patient age, and the catheter location. “Lactate/pyruvate ratio was considered an extremely useful marker of cerebral stress” and the trend was used to guide the subsequent therapy.

Through careful monitoring of glucose data they observed that a tight glycemic control, and thus a lower level of brain glucose as measured by microdialysis, resulted in higher lactate/pyruvate ratios. With a transition to loose glycemic control parameters, the brain glucose increased to the normal range and the lactate/pyruvate ratio dramatically decreased. Similar findings have been reported by Vespa *et.al.*(2003) and Oddo *et.al.* (2008).

CONCLUSIONS

Forty years ago (1972), at the Karolinska institute, we implanted the first microdialysis probes into rat brains (Ungerstedt and Pycock 1974). Twenty-five years ago (1987), we implanted the first microdialysis catheters in Parkinson patients undergoing thalamotomy to relieve tremor (Meyerson *et.al.* (1990).

Publication was not easy. Nature turned down the first paper with the argument that “anyone can understand that you get out dopamine if you place a dialysis tube in the striatum of a rat” and the first human studies raised a number of ethical issues. Today we know that microdialysis functions like a “universal biosensor”, which is able to extract essentially any chemical substance from the extracellular fluid of the brain. In comparison it causes less damage to brain tissue than both ventricular catheters and parenchymal pressure sensors.

During neurointensive care microdialysis is the only technique able to deliver information about changes in brain metabolism over time in response to clinical interventions. When a microdialysis catheter is combined with an oxygen sensor the intensivist can follow the delivery of the two essential brain nutrients, glucose and oxygen, and the resulting level of energy metabolism in absolute numbers i.e. the lactate/pyruvate ratio.

Although the relationship between microdialysis data and patient outcome is well established in the literature there are two factors determining the success of bedside microdialysis: (1) The placement of the microdialysis catheter in the tissue at risk and (2) the willingness to individualize patient treatment with the intention of normalizing brain chemistry.

The staff must be attentive and prepared to act on such changes in brain chemistry that are early warnings of secondary insults. At the same time they must respond to the effects of their therapeutic interventions on brain metabolism.

Our obvious aim is to alleviate ischemia caused by the primary insult and avoid secondary ischemia during the course of treatment. Elevation of the lactate/pyruvate ratio and a decrease of glucose are early

warnings of insufficient capillary perfusion i.e. ischemia, leading toward mitochondrial dysfunction. An increase in glycerol is an indicator of cell membrane damage. We can improve capillary perfusion by actively individualizing CPP and secure adequate supply of oxygen and glucose by adjusting oxygenation and blood glucose.

Looking into the future I am confident that we will see new drugs protecting mitochondrial function, increasing capillary perfusion, stabilizing membranes, repairing the blood brain barrier etc. **Until then: Keep responding actively to microdialysis data with all the well-known interventions that are available already today.**

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