QualiTest

Appeal

Coagulation Diagnostics

The influence of temperature and acid-base balance on coagulation and fibrinolysis must be taken into consideration in diagnostics

Editorial

If diagnostics and therapy lag behind the state of the art, it is hopefully just economical constraints of the medical technology and pharmaceutical industry which are responsible. In this case, the doctor who uses diagnostic and therapeutic products has to make an appeal to those companies to eventually act in the sense of progress.

A current example is an unusual appeal to provide an optimal solution for infusion in the field of pediatrics: "Medical companies, please provide us with this special perioperative infusion fluid as it will definitely have the potential of saving lives!" [10].

A similar appeal is made in this edition of QualiTest to companies operating in the field of coagulation diagnostics in the broadest sense, i.e. near patient testing (point of care, POC) as well as laboratory diagnostics, to improve their products.

It should no longer be ignored that such products have a couple of marked defaults. Concretely speaking: In addition to the temperature, the influence of the acid-base balance on a patient's coagulation and fibrinolysis has currently become so important that survival of patients with major bleeding can be ensured only if any acidosis is prevented: coagulopathy, metabolic acidosis and hypothermia are nowadays considered to be a lethal triad for the patient [9]. More concretely speaking, the coagulation disorder due to hypothermia and acidosis limits survival. The diagnostics of coagulation therefore reached a significance which is not restricted to the detection of disorders such as hypo- and hypercoagulation, but should include assessment of the success of pharmacotherapy. Therefore, this appeal is also made to companies offering coagulation therapies. RZ

No. 11
October 2009

Code

Particularly high demands have to be made on medical diagnostic systems as far as safety and functionality of the equipment are concerned on the one hand and the accuracy and reliability of the findings obtained with such equipment on the other hand. These are decisive criteria influencing the diagnostic and therapeutic action that is taken by the doctor for the benefit of his/her patients.

The requirements can be met only if the finished equipment is subjected to continuous objective internal and external quality control.

Moreover, economy dictates that the cost of implementation of the equipment, of continuous maintenance and quality control in relation to the expected diagnostics and possible treatment is favourable.

Deliberate acceptance of deficiencies in quality to the detriment of the patient represents a limit in the competition between manufacturers.

Maximum transparency with regard to the type of test conducted, the declaration of a 'gold standard', the selected examiners as well as the published results is a prerequisite for successful external quality control by a test lab.

For the sake of fairness, the manufacturer or seller concerned is given the opportunity to make a statement in each QualiTest publication if they previously commissioned the test lab to carry out an evaluation.

The test laboratory can only pursue its activities successfully if the operator, employees and examining experts on the one hand and the customers on the other are able to identify with this code.

Physioklin

Pleading in the Field of Coagulation Diagnostics

Arguments for Improving Coagulation Diagnostics

Background

In addition to temperature, the influence of the acid-base balance on a patient's coagulation and fibrinolysis has nowadays become so important that survival in patients with major bleeding can be ensured only if any acidosis is prevented: coagulopathy, metabolic acidosis and hypothermia are nowadays considered to be a lethal triad for the patient [9]. Since hypothermia and acidosis cause coagulopathy, this is, strictly speaking, not a triad. Hypothermia and acidosis alone are fatal because they can provoke exsanguination of the hemorrhagic patient.

Acidosis

We would like to present two findings regarding acidosis:

- The relation between **activity or activation of various coagulation factors and the pH or BE value** in vitro [14] is represented in Fig. **1**: A negative BE of approximately –15 mmol/l reduces the activity/activation of various coagulation factors to approximately 50 %.
- Relation between **prothrombin time and BE value** according to Fig. **2**: In more than 4,000 **seriously injured** polytrauma patients, there is a highly significant relation between prothrombin time (PT) and BE. If the BE is approx. –15 mmol/l, the PT falls to approx. 50 %.

Alkalosis

On the other hand, there is an increased coagulation with a risk of intravascular coagulopathy (thrombosis), if the pH increases towards alkalosis:

If the pH is 7.60 (base excess 16.5 mmol/l), the activity/activation of the various coagulation factors is reported to be twice as high [14].

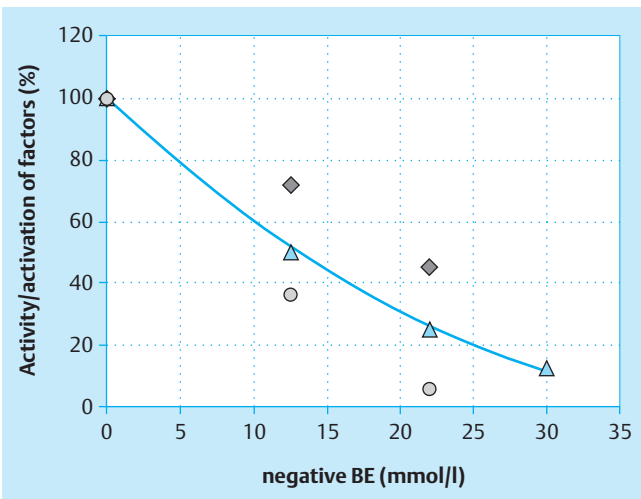

Fig. **1** pH dependence of selected coagulation factors in vitro (Δ activation of IIa, ○ VIIa activity, ◊ activation of Xa) after transformation to BE values (mmol/l), assuming a nonrespiratory acidosis [14]. A BE of approximately –15 mmol/l reduces the activity/activation of various coagulation factors to approximately 50 %.

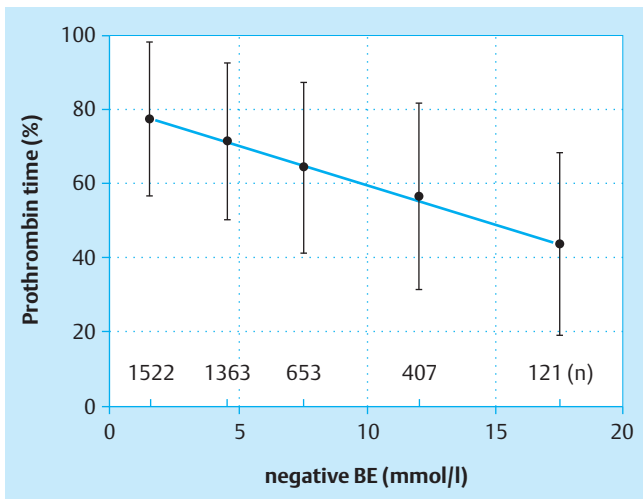

Fig. **2** Highly significant ($p < 0.001$) correlation between prothrombin time (%) and the negative base excess of blood (BE, mmol/l) in 4,066 out of a total of 20,815 seriously injured (ISS > 16) polytrauma patients undergoing primary care from 1993 to 2004 from the Trauma Registry of the German Society of Trauma Surgery (DGU) [after Lefering and Rixen, DGU 2006]: If the BE is approx. –15 mmol/l, the prothrombin time is reduced to approximately 50 %.

Diagnostics

Problems

The boundary conditions must be taken into consideration when carrying out diagnostic testing of coagulation and fibrinolysis. This holds true for temperature as well as for the acid-base balance of the sample.

Hypothermia

The problem can be described using the example of hypothermia, i.e. a patient's reduced body temperature. The coagulation function of a patient with a body temperature of 32 instead of 37 °C is reduced by approximately 50 % due to the mere hypothermia. If coagulation diagnostics were performed, e.g. by thromboelastography (TEG) at 37 °C, an erroneous diagnosis would be generated, because the blood sample of the hypothermic patient, which is returned to normal temperature in the instrument, would mimic a normal coagulation status.

Acid-base balance

The same statement holds true for the patient's acid-base balance, defined by his/her pH in connection with the BE (base excess or base deficit, mmol/l) as well as the carbon dioxide partial pressure (pCO_2, mmHg) of his/her blood.

If the pH is 7.20 (base deficit 12.5 mmol/l), the coagulation activity is reduced by half and if the pH is 7.60 (base excess 16.5 mmol/l), the coagulation activity is doubled. If coagulation diagnostics are performed in such a way that changes of the pH or BE level are offset during diagnostics (e.g. by using buffered reagents) or that changes of the pH are admitted (if the pH of a sample, e.g., increases towards alkalosis as a consequence of CO_2 loss), a coagulopathy caused by acidosis cannot be detected any longer or increased coagulation may be feigned.

It may be assumed that many of the published contradictory findings (see below) can be explained by unintentional changes of the acid-base balance, pH, BE and pCO_2.

Examples of Obvious or Suspected Errors of Previous Diagnostics

1. Patient with hypothermia

If the blood sample of a hypothermic patient is carried out under normal temperature conditions in the instrument, the coagulation status is seemingly normal. Therefore, the patient temperature can nowadays ideally be set in point of care instruments for TEG. Due to the considerable temperature dependence, this was also requested very early for the determination of PT and PTT [15].

If it is, e.g. currently pretended for TEG that the acidosis produced in vitro alone has no effect on coagulation, but only synergistically together with hypothermia [2], this finding should be repeated with an optimized TEG method under traceable conditions of the acid-base balance of the samples.

2. Determination of PT using buffered reagents

Our own in vitro experiments on a possible relation between PT and base excess of a blood sample [21a] using Thromborel-S reagents (Dade Behring) or buffered Hepato-Quick reagents (Roche Diagnostics) demonstrate that there is a marked effect of the BE on the prothrombin time when using "traditional", i.e. unbuffered reagents, which is largely "covered up" when using buffered reagents.

A corresponding remark was published earlier [21a]: An enquiry made to Roche Diagnostics (Mannheim, Germany) why their reagents (Hepato Quick) used buffered diluents, could, unfortunately, not be answered conclusively.

3. Preanalytics have an influence on the results of diagnostics

Any possible modifications of the pH, BE and pCO_2 are to be prevented during the diagnostic process if a patient's current (!) coagulation diagnostics are to be performed correctly. If e.g. the pH of the sample rises due to a CO_2 loss in the sense of alkalosis, hypercoagulopathy can be mimicked or an acidosis-related hypocoagulopathy may be masked.

Examples:

Sampling errors (CO_2 loss to different extents, alkalosis), venostasis during blood sampling (acidosis of blood), arterial and venous blood produce different results (different pH values), the use of butterfly systems (free drain of blood) may change the findings.

Pipetting error, incorrect fill volume of the tube (CO_2 loss, alkalosis), incorrect mixture ratio of blood/citrate (dilution acidosis of various degrees).

4. The methodology has an influence on the results of diagnostics

The above mentioned facts can be applied to the actual diagnostics as well.

Examples:

A blood sample, and all the more a plasma sample in contact with ambient air, e.g. in the TEG (Rotem), undergoes withdrawal of CO_2 by diffusion. The reduction of the carbon dioxide partial pressure (pCO_2) from a normal value of 40 mmHg (arterial blood) or approx. 50 mmHg (venous blood) to lower values lead to alkalinization with an increase of the pH value above the normal value of 7.40. This holds particularly true for activities such as centrifugation used to obtain plasma, mixing of samples with plasma expanders, addition of CO_2-free reagents etc. A too large volume of reagents provokes a pH reduction; if the reagents added are buffered, they will change the original pH value of the sample and thus the result.

5. Supposed effect of solutions for infusion

It was investigated in numerous studies whether solutions for infusion have an in vitro or in vivo effect on coagulation or not. The in vitro methods used, which may have been insufficient, may have mimicked such effects. If, e.g., blood samples are mixed with HCO_3^--free solutions, such as 0.9% NaCl in a ratio of 1 + 1, this will lead to dilution acidosis, the pH will fall from 7.40 to 7.10 [20] which will result in a supposed coagulopathy *in vitro*.

6. Hypercoagulopathy due to hemodilution?

It is said that in vitro hemodilution with 0.9% NaCl, lactated Ringer's solution (cave: lactate is only effective in vivo) or electrolyte solution lead to an increased coagulation in the sense of hypercoagulopathy [3, 7, 16, 18]. In practice, it looks like this [7]: Blood is centrifuged for 30 min, RBCs and plasma are separated and new hematocrits (10, 20, 30 and 40%) are set; each reduction of the hematocrit accelerates coagulation, probably because the pH increase is continuously enhanced due to the CO_2 withdrawal.

Doubts arose about such TEG findings, because hypercoagulopathy does not occur if hemodilution is made with cerebrospinal fluid taken from the patient by spinal puncture [18]. In fact, this physiological fluid contains HCO_3 and has a normal pCO_2, which easily explains the lack of hypercoagulability.

It remains unclear why hemodilution should lead to coagulation activation [5] and can possibly be explained by methodological problems [11]. A dilution hypocoagulopathy would actually be more likely.

7. Supposed coagulation effects of colloids

Colloids in solutions for infusion are attributed to supposed coagulation effects, which are called into question for methodological reasons (TEG): The primary in vivo hemostasis is said to be inhibited by gelatin [1] and to lead to dilution coagulopathy in animal experiments [6], HES, gelatin and albumin are said to interfere with coagulation in vitro [3] and HES is supposed to cause hypercoagulopathy [17] or hypocoagulopathy [12] in vivo depending on the author.

Consequences for Diagnostics

It becomes evident that there are two different demands on optimized coagulation diagnostics, namely avoidance of errors which

1. have an influence on the result, i.e. either to "dress it up", e.g. CO_2 loss (alkalosis, hypercoagulopathy), or "deteriorate" it (dilution of sample, acidosis, hypocoagulopathy), the demand being the maintenance of present conditions, including the phase of preanalytics, and
2. reverse the sample's history, e.g. measuring at 37 instead of 32 °C (offsetting hypothermia) or use of buffered reagents (offsetting acidosis), i.e. change from in vitro conditions to "theoretical" in vivo conditions.

Regarding item 2, it might be considered to introduce a "temperature correction in vitro → in vivo" from hypo- to normothermia, as soon as evidence of the accuracy of such corrections has been provided in experiments. This seems, however, impossible for the acid-base balance in the near future.

Possible Effect of Current Diagnostics on Coagulation Therapies

The shortcoming of coagulation therapies is that their effect is based on a normal acid-base balance, especially BE.

1. rFVIIa (NovoSeven)

Metabolic acidosis is considered as exclusion criterion in international recommendations on the use of recombinant coagulation factor VII a (rFVIIa, NovoSeven) [13, 19]. A base deficit of 12.5 mmol/l (or pH 7.20) is generally defined as the limit. The recommendation even stated that prior to the use of the product, the pH should be brought "as near as possible to the physiological level" [19].

2. Products modifying fibrinolysis

Since the coagulation activity strongly depends on the pH, it can be assumed that fibrinolysis and products modifying fibrinolysis, e.g. aprotinin (Trasylol) or tranexamic acid, are influenced by the BE as well. The BE may have been an efficacy-lim-

iting factor related to the decision that aprotinin had to be taken from the market [4].

How Could Coagulation Diagnostics be Optimized?

Optimal *in vitro* coagulation diagnostics should rule out changes of parameters of the sample (plasma, whole blood) which are relevant for coagulation, i.e. temperature and pH with CO_2 partial pressure and base excess, and thus leave the corresponding in vivo patient data unchanged. Such in vitro diagnostics primarily warn the doctor with a negative finding and prevent any false positive (normal) coagulation status. Secondarily, a negative finding requires the doctor to perform additional diagnostics regarding the causes. This may refer to temperature and acid base status and require a correction before any further diagnostic and therapeutic steps are considered.

Purpose of this Appeal

The purpose of this appeal is to call on medical technology companies involved in coagulation diagnostics in the broadest sense, i.e. near patient diagnostics (point of care, POC) as well as laboratory diagnostics, because it is high time to improve their products. The findings repeatedly published since 2003 cannot be negated any longer [14, 21a–d]. The advantage of POC diagnostics is that the parameters with influence on the diagnostic procedure, i.e. temperature and acid-base status (pH, BE, pCO_2), can be taken into account at the point of care.

Addressees

The following (incomplete) list represents examples of companies addressed, because they provide instruments and procedures for the coagulation and fibrinolysis diagnostics including platelet function diagnostics (for central laboratories or POC testing):

Abbott (i-STAT), Dade Behring (PFA-100 Analyzer), Dynabyte (Multiplate Analyzer), Haemonetics (Haemoscope, TEG), Haemachem (St. Louis, USA) (Heptest), ITC (Edison, USA) (Sur-gicutt, Hemochron), Pentapharm (Rotem), Sienco (Arvada, USA) (Sonoclot), SycoMed (ball or hook coagulometers), Roche Diagnostics (CoaguChek).

This appeal also addresses pharmaceuticals companies offering coagulation therapies, because such companies will have a great interest in the availability of optimal diagnostics for the assessment of success and monitoring of treatment.

As examples, the following companies are addressed (incomplete list):
Baxter, Bayer Healthcare, Biotest, Boehringer Ingelheim, CSL Behring, Novo Nordisk, Octapharma, Pfizer Pharma, Wyeth.

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c. 05.08.2008 – Major bleeding – Prevent acidosis!
d. 18.12.2008 – Alter von Erythrozyten-Konzentraten

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Impressum

QualiTest® is published irregularly at www.Physioklin.de.

QualiTest No. 11, October 2009, is enclosed to the "Transfusion Medicine and Hemotherapy" journal (06/09) (Verlag S. Karger) (print run 3000).

Editing and copyright
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The Physioklin Test Lab was established in 2007 from the third-party funded project Hemodiagnostics Test Lab at the Institute of Physiology and Pathophysiology of the University of Mainz. It finances itself by producing expert reports for equipment manufacturers. By independent function testing and quality control of hemodiagnostic equipment its general aim is to test, improve and guarantee the quality of such equipment and to publish the corresponding results. Examining experts may only – with or without payment of a fee – work for the test lab subject to agreement to declare any possible conflict of interests. This could arise if the examining experts receive material or financial contributions from the manufacturer or seller of the equipment or method concerned.